

Clean form of amended paragraphs in Example 17 of U.S. Serial No. 09/536,736 at p. 34, line 27-p. 35, line 30 of the specification:

According to Example 3, plastic columns were assembled with commercially available membranes (Pall Gelman Sciences, Hydrolon with a pore size of 1.2 or 3 μm ; Sartorius, SARTOLON® polyamide filter membrane with a pore size of 0.45 μm).

For isolation of RNA, two different starting materials were used:

- 1) total RNA from liver (mouse) in an aqueous solution; the purification of total RNA and the elution were carried out as described in Example 4; and
- 2) 5×10^5 HeLa cells, the purification of total RNA and the elution were carried out as described in Example 3.

For each test, 20 ng of isolated total RNA were used. As a control, RNA purified using RNEASY® RNA isolation-Kits (Qiagen GmbH) and a sample without RNA were used.

AI A RT-PCR was performed with these samples under standard conditions. For amplification two different primer pairs were used for the β -Actin. A 150 Bp-sized fragment served as proof of sensitivity, a 1.7 kBp-sized fragment assessed the integrity of the RNA. From the RT-reaction, 1 μl was removed and transferred to the subsequent PCR. 25 cycles were performed for the small fragment and 27 cycles for the large fragment. The annealing temperature was 55°C. The amplified samples were subsequently placed on a non-denaturing gel and analyzed.

For the 20 ng volume used of total RNA isolated in the process described above, the corresponding DNA-fragments can be demonstrated in the RT-PCR. When using total RNA from mouse liver, no transcript can be demonstrated, as the conditions used here were adjusted to human β -Actin. The control specimens which contain no RNA did not produce any signals.

Figure 7 shows ethidium bromide stained gels of an electrophoretic separation of RT-reactions.

Figure 7A: Lane 1 to 8: RT-PCR of a 150 Bp-fragment;

Lane 1, 2: RNA from an aqueous solution purified with the Hydrolon 1.2 μm membrane;

Lane 3, 4: RNA from HeLa cells purified with the SARTOLON® polyamide filter membrane;

Lane 5, 6: RNA from HeLa cells purified with the Hydrolon 3 μm membrane;

Lane 7: RNA purified by way of RNEASY® RNA isolation-Mini-Kit;

Lane 8: Control without RNA.

Figure 7B: Lane 1 to 8: RT-PCR of a 1.7 kbp-fragment;

Lane 1, 2: RNA from an aqueous solution purified with the Hydrolon 1.2 μm membrane;

Lane 3, 4: RNA from HeLa cells purified with the SARTOLON® polyamide filter membrane;

Lane 5, 6: RNA from HeLa cells purified with the Hydrolon 3 μm membrane;

Lane 7: RNA purified by way of RNEASY® RNA isolation-Mini-Kit;

Lane 8: Control without RNA.
